

FROM THE MEDICAL CLINIC OF THE UNIVERSITY OF ZÜRICH

Director: Professor Dr. Wilhelm Löffler

Work under the direction of P.-D. Dr. S. Moeschlin

**The Effect of Demecolcin (Des-methyl-
colchicine) on the Peripheral Blood and
Bone Marrow in the Rabbit and Cat**

INAUGURAL-DISSERTATION

for the Degree of Doctor of Medicine
from the Medical Faculty of the University of Zürich

Presented by

Alvin Lichtman

of Stamford, Connecticut, U.S.A.

Accepted on the Proposal of Prof. Dr. W. Löffler
and P.-D. Dr. S. Moeschlin

Zürich 1954
Buchdruckerei Fluntern
Plattenstrasse 27

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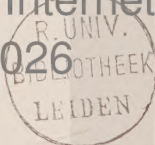
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TO MY PARENTS

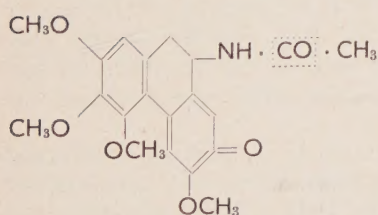
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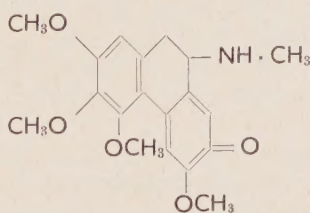
I. INTRODUCTION

Since Jacoby (9) first described the effects of Colchicine in arresting the mitosis of cells, there have been numerous attempts to utilize this drug in the therapy of diseases with altered mitotic activity as in malignant tumors and blood diseases. However an important hinderance were the disagreeable side affects of the drug precluding the attaining of a sufficiently high therapeutic concentration in the blood. Attempts to overcome this by injecting the drug directly into the tumor or into the medullary cavity were similarly thwarted by the rapid dissipation of the drug into the blood stream. Some results were obtained by this method but they were merely transient. Santavy and Reichstein (22) have now managed to isolate one of the constituents of Colchicine in purified form and thereby believe to have eliminated some of the undesirable side affects so that higher blood levels can be obtained.

This drug, Desmethycolchicine (=Demecolcine), was kindly placed at our disposal by the Ciba Drug Corp. of Basel, Switzerland to *investigate the therapeutic dosage and its effect on the peripheral blood and bone marrow in rabbits and cats*. In the drug, a methyl group is substituted for the acetyl group normally present.



Colchicine



Demecolcine

II. LITERATURE

After the original work of *Jacoby* (9) showed that colchicine arrested cell mitosis, particularly that of the most active mitotic cells in the body, considerable interest was aroused as to its possible use in tumors and studies of cell activity.

Maldin and Dixon showed that it had a depressing effect on the activity of the bone marrow. Although considerable work has been done (with reference to therapy of tumors), it will not be discussed in this paper because it lies without its scope.

Levison (12) working with small doses of 1—2 mgm. observed that after a latent period of 72 hours there was a rise in the number of neutrophils present in the peripheral blood.

Beck (3) used doses of 0.1 mgm. per kilogram of body weight which produced a fall in the leucocyte count within 2—6 hours and a subsequent rise in the neutrophils on the second day to 50% above the initial values. He noted also that the colchicine had a cumulative action requiring 8 days for excretion.

Vollmer (25) showed that the mortality of small experimental animals was inversely proportional to their weight with death occurring within 36 to 73 hours after dosage.

Loicq (14), working with single doses of 3 mgm. per kilogram of body weight, observed an initial leucopenia which changed to a leucocytosis within 8—12 hours. There was an increase in the coagulation of the blood during both these states. Further work by later authors supported these findings.

Balduini and Bertolotti (2) tried both acute intoxications with single doses of 5—7 mgm. per kilogram of body weight, and chronic effects with 0.60—0.65 mgm. per kilogram for 20 to 25 days. There was an initial leucocytosis with increased neutrophils which progressed into a leucopenia which was due to a drop in the neutrophils. There was a relative rise in the lymphocytes but it was only a small absolute rise. The white cell elements showed a slight shift to the left while the red cells had a right shift. A prevalence of mitotic cells in the prophase was noted.

Widmann (26) observed an initial drop in the leucocytes followed by a sharp rise particularly in the neutrophils. There was an exhaustion of the marrow with a loss of mature cells. Only a light toxic effect was seen on the lymphocytes.

Albrecht and Boll (1) working with marrows observed an arrest in the mitosis of the marrow in early metaphase. This agrees with the work of *Bucher* (5), who demonstrated that colchicine stops mitosis in the metaphase. Similar effects were recorded in cases of accidental poisoning in humans caused by the ingestion of the seeds of the autumn crocus, by *Dustin* (6) and by *Seifert* (24).

These results promoted the clinical trial of colchicine in the treatment of leukemias.

Paul, Brown and Limarzi (20) tried it in a case of chronic myelogenous leukemia but did not feel the patient was beneficially affected. Autopsy showed leukemic cells with obvious changes characteristic of colchicine but not typical for the drug. Toxic doses, as high as 48 mgm., were used resulting in depression of the erythrocytes and hemoglobin.

Kneedler (10) used the drug in a case of acute myelogenous leukemia. The patient alternated between acute and chronic stages for 13 and one half months before expiring in a typical leukemic death, however, it was felt that the course of the disease had been modified. An important drawback to the therapy observed in these cases was that when therapeutically active doses were attained, the side effects which ensued prevented continuance of the drug at these levels. These effects were pyralism, muscular pains, nausea, vomiting, and diarrhea (*Moeschlin* [16]). Some clinicians attempted to overcome this by injecting the drug directly into the medullary cavity, but the drug was soon absorbed into the blood so that therapeutic doses could not be maintained.

Guichard, Brette and Phillippe (7) and earlier *Bernard* (4), tried this method but their results were only suggestive so that they felt more work was necessary.

Obviously, if a colchicine preparation could be found which retained its mitotic retarding activity while its toxic effects were diminished, an important advantage could be gained.

Santavy and Reichstein (22) succeeded in deriving certain pure alkaloids from the raw colchicine which warranted testing. Among these desmethylcolchicine, in which the normal acetyl group of colchicin is replaced by a methyl group (see page 5), exhibited a mitosis depression similar to normal colchicine but had a lethal i.v. dose 30—40 times higher than normal (*Schär, Loustalot and Gross* [23]). These findings prompted the undertaking of the present set of experiments on rabbits and cats to determine just what its effect would be on the hemopoietic tissue of these animals and its possible clinical use in leukemias.

III. EXPERIMENTAL STUDY

Materials and Methods

A total of 17 rabbits and 6 cats were used in this study. The rabbits were kept separately in cages in the same room as the cats, who were allowed to roam freely in the room. The rabbits were kept on a turnip diet, and the cats were fed meat and milk. The rabbits were divided into four groups. Group I comprised 5 normal, untreated animals used as

controls; Group 2 consisted of 3 animals who received 3 mg. of desmethylcolchicine per kilogram of body weight for a period of 23 days; Group 3 comprised 12 animals who received 3 mg. of desmethylcolchicine per kilogram of body weight for a period of 14 days; Group 4 consisted of 2 of the control animals who were subsequently used to test the efficiency of the oral administration of the drug with a dose of 3 mg. per kilogram of body weight. The cats were divided into two groups. Group 1 contained 2 normal, untreated animals maintained as controls; Group 2 consisted of 4 animals who were first administered $\frac{1}{2}$ mg. of the drug per kilogram, but this was modified to $\frac{1}{4}$ mg. when it became apparent that it was too toxic.

In preliminary experiments in rabbits it was found that 3.0 mg. per kilogram of desmethyl colchicine would produce a definite change in the peripheral blood picture without being unduly toxic. This was found to be completely too high for the cats who were much more sensitive and although an initial dose of $\frac{1}{2}$ mg. per kilogram seemed adequate, it subsequently proved to be toxic and had to be lowered to $\frac{1}{4}$ mg. per kilogram of body weight.

Technique of Desmethylcolchicine Administration:

Three milligrams of desmethylcolchicine per kilogram of body weight were slowly injected into the marginal vein of the rabbit from ampules of prepared solution containing 1 mg. of the drug per 1 c.c. of saline solution. The cats were lifted by the scruff of their necks and injected with the proper dosage intraperitoneally through a shaved area on the abdomen.

Hematological Studies. — Peripheral blood was obtained from the small veins in the ears of the animals prior to administration of the drug in order to avoid any immediate affect in the blood picture. Studies, using standard techniques, included the following:

- a) *Erythrocytes* per cubic millimeter and Hemoglobin in grams per 100 cubic centimeters were recorded every 3 days.
- b) *Reticulocytes* in per cent of erythrocytes were determined once a week.
- c) *Total leucocytes* per cubic millimeter and differential smears were done daily on the treated animals and every 2 days on the controls.

Bone Marrow Studies. — Narconumal was administered intravenously to the rabbits in a dosage of $\frac{3}{4}$ mg. per kilogram of body weight to induce anesthesia sufficient for the marrow puncture. The marrow was obtained by aspiration of the medullary cavity in the upper end of the tibia. Marrow studies were done prior to, and at the end of the drug administration, and also when the peripheral blood had returned to normal after cessation of the drug. Similar studies were

undertaken on the cats with the only modification of $\frac{1}{3}$ mg. of Narconumal per kilogram of body weight injected intraperitoneally to induce anesthesia. The controls underwent a similar series of marrows. The differential enumeration of the marrow cells was made according to the method of Rohr, in which the number of nucleated and reticulum cells per 100 white cells are counted separately. A total of 300 to 500 cells was counted in each marrow study.

Weight Studies. — The animals were weighed once a week and any variations recorded.

Histopathologic Studies. — No animals were specifically sacrificed, but any animals that died were examined grossly and microscopically to observe any affects of the drug on the main tissues.

Experimental Results

A. Rabbits

1. Blood Changes

In all a total of 11 rabbits were observed under a dosage of 3 mg. of Desmethylocolchicine, if we disregard the 4 rabbits who died under the course of medication. They will be discussed later. Under the influence of the drug a marked reduction occurred in the total number of leucocytes which was mainly a reflection of a profound reduction in the number of neutrophils.

Latent Period in Effect: This was found to vary according to the method of administration of the drug. In the animals who received the medication intravenously, the drop in the leucocytes was noted to begin on the second day and reach its maximum in 8 to 10 days (see fig. 1). These values were constant to within a day on the animals observed. Since the drug could be given orally also, two animals were taken from the control group and given 3 mg. per kilogram of bodyweight to see if any difference in effect existed. Here the latent period was found to have been considerably increased being 12 to 14 days, but then the subsequent changes in the blood and the reaching of a maximal drop in the leucocytes were similar to the previous observations (see fig. 1).

Renewed Rise of the Cells: Within a day after the discontinuance of the drug, the leucocyte values began to rise reaching the initial cell values within 8 to 10 days.

Neutrophils: The most profound effect produced in the peripheral blood picture was in a sharp drop in the Neutrophils. During the course of therapy they dropped to a level of $\frac{1}{4}$ of the initial value. In one animal they were observed to drop from an initial value of 4,000 per cubic mm. to a level of 300 per cubic mm. An occasional metamyelocyte was observed in the peripheral blood during the drug administration, but more often after cessation of the drug. Tendency to hypersegmentation.

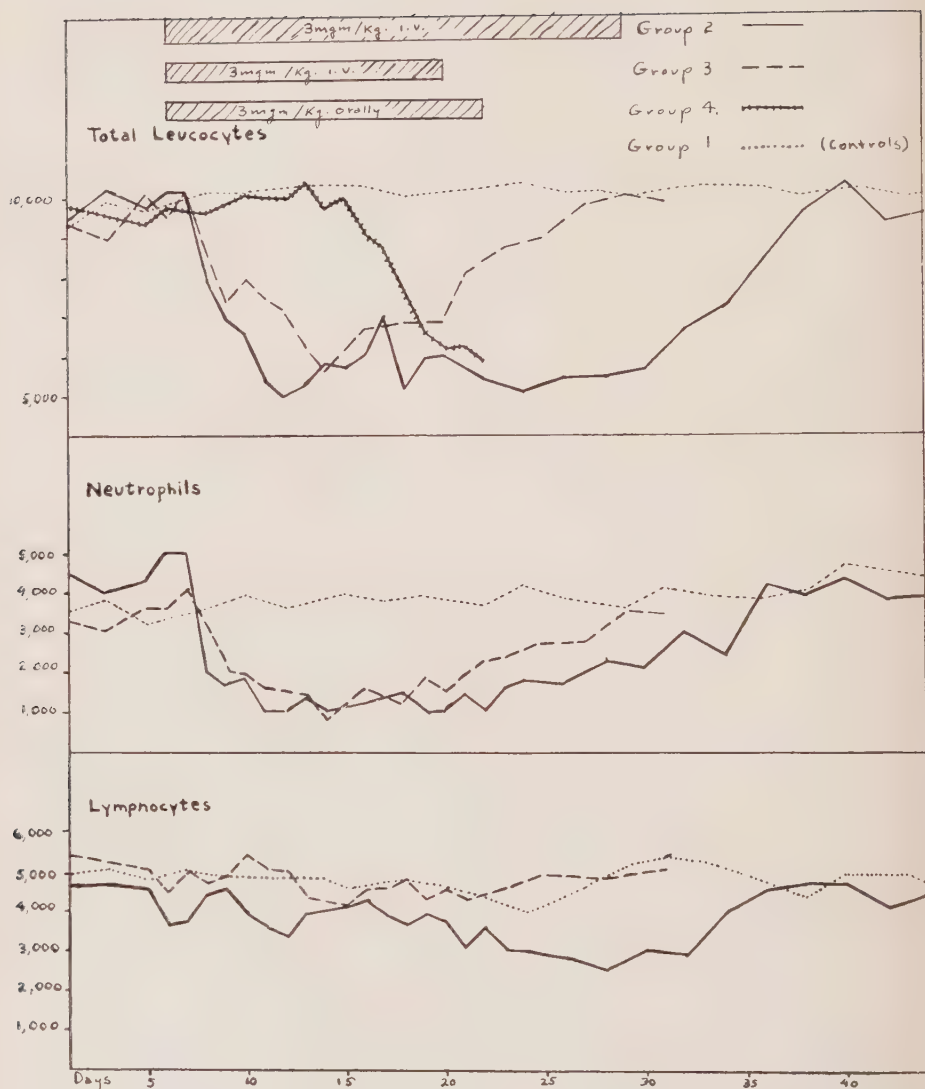
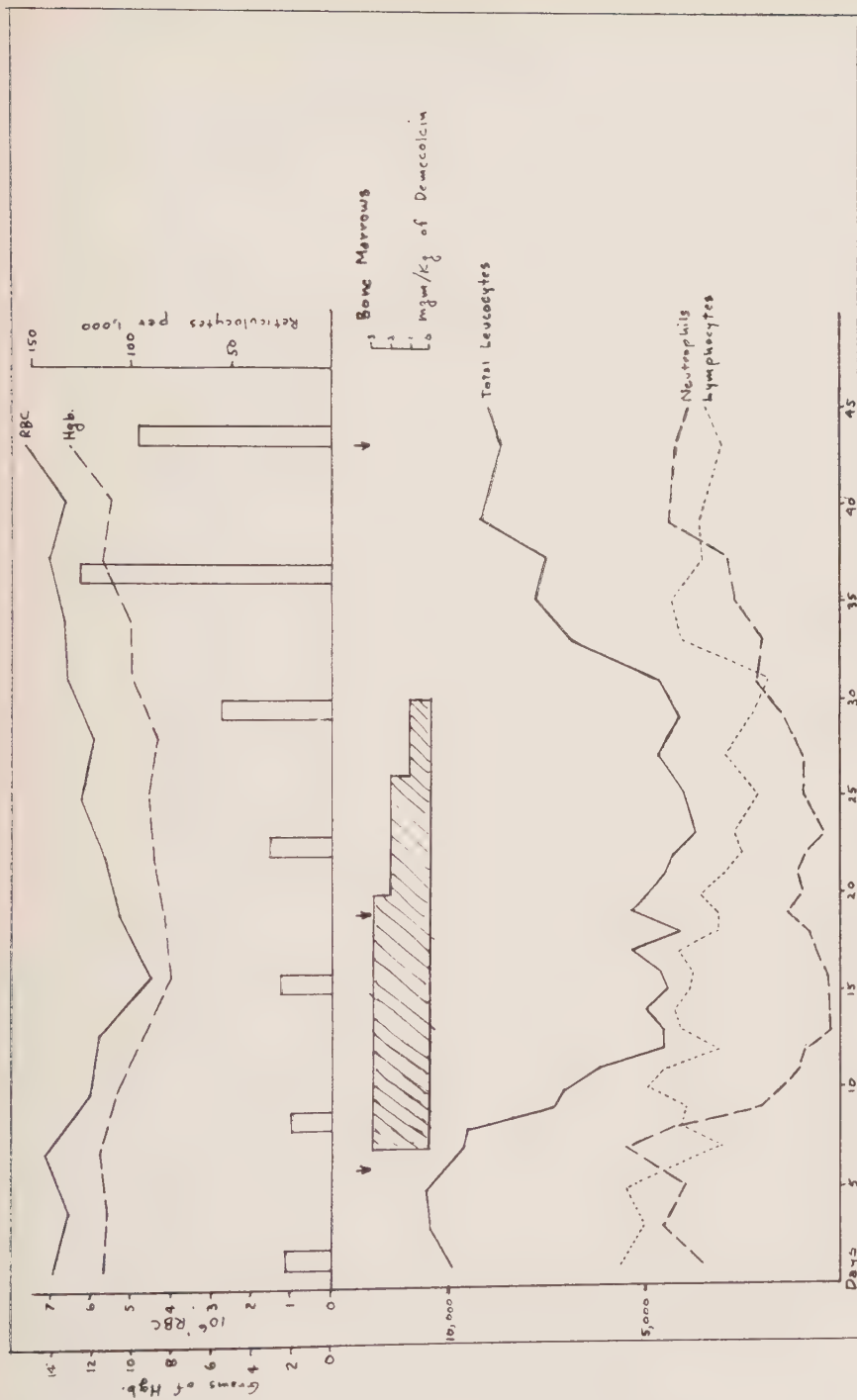


Fig. 1a



Typical Rabbit

Fig. 1b

Lymphocytes: The behavior of the lymphocytes was particularly interesting since they were relatively unaffected during the course of therapy. In Group 3, where the animals had a short run, no change was noted at all although the Neutrophils and as a result the total leucocyte count dropped. In Group 2, where the animals were maintained on the drug for a longer period of time, there was a small drop in the Lymphocytes.

Eosinophils, Monocytes, and Basophils: Showed no marked change during the therapy although they were inclined to show some decrease.

Thrombocytes: No absolute count of the thrombocytes was made during the experiment but they were checked on the smears and found to be plentiful and agglutinated in groups during the entire course of treatment.

Erythrocytes: The erythrocytes showed no striking fall although there was a slight drop in general and as much as 2 million erythrocytes in a few of the animals but this generally reflected a higher toxicity and was accompanied by a drop in the lymphocytes as well. It is also significant that the only animal showing a profound drop in the erythrocytes to 3.5 million from an initial value of 7 million died on the 9th day of treatment. Normocytes were observed in the peripheral blood during the therapy.

Reticulocytes: During the course of therapy no significant change in the number of reticulocytes was observed *but after cessation of the drug, a sharp increase was seen with a rise from an average of 26 reticulocytes per 1,000 to values as high as 234 reticulocytes per 1,000.*

B. Cats

1. Blood Changes

In all a total of 4 cats were observed under treatment with Desmethylocolchicine in addition to two control animals. As a result of preliminary experimentation, the dosage was set at $\frac{1}{2}$ mg. per kilogram of body weight, but this was found to be too toxic and had to be lowered to $\frac{1}{4}$ mg. per kilogram of body weight during the course of the experiment. Although 4 animals were started out as a single group, two of the animals developed such severe side affects that the drug was discontinued after one week and they are therefore recorded as Group 2. The remaining two animals were not as severely affected and the dosage was then modified to $\frac{1}{4}$ mg. for an additional week.

Latent Period in Effect: Although the cats received the drug intraperitoneally instead of intravenously as the rabbits had, the drop in leucocytes was again found to start on the second day and reached a maximum on the sixth day (see fig. 2).

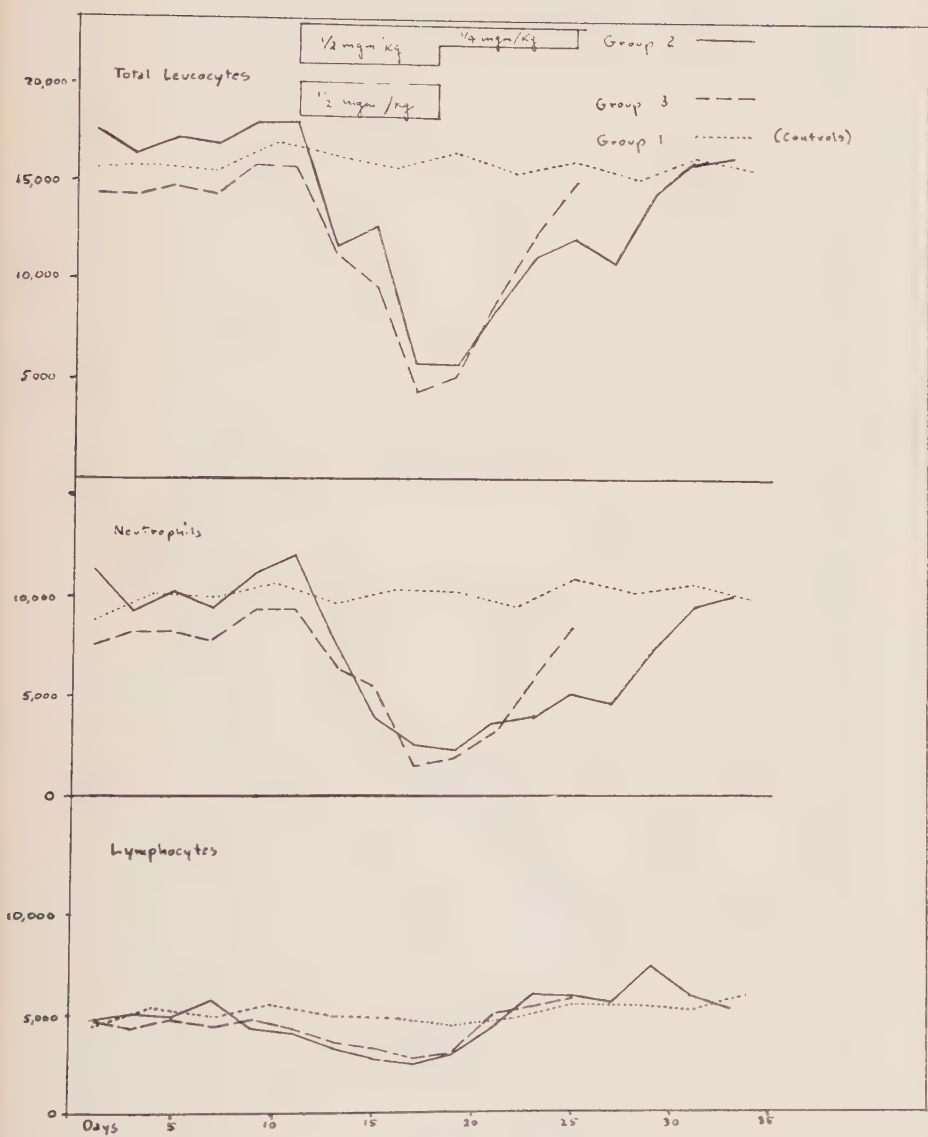


Fig. 2



Fig. 2 b

Renewed rise of the Cells: Within two days after the discontinuation of the drug, the leucocytes began to show a rise, reaching their initial values in a period of 6 to 8 days.

Neutrophils: A sharp drop in the number of neutrophils was seen starting within 2 days after the beginning of administration of the drug. There was a dramatic drop from an average of 9,000 per cubic mm. to a level of 2,000 neutrophils per cubic mm. *The cells tended to show over-segmentation* although an occasional metamyelocyte could be seen. *After the drug was stopped, there was a rapid rise with a tendency toward stab cells.*

Lymphocytes: Although there was no marked decrease as observed in the neutrophils, the lymphocytes showed a definite decrease during the course of therapy. Since this was observed in the rabbits it indicates a manifestation of the toxicity observed in the cats. After the drug was discontinued or merely lowered in Group 3, there was an immediate rise in the lymphocytes even surpassing the original values.

Erythrocytes: The erythrocytes showed no significant change in number inspite of the toxic manifestations. There were a number of normocytes in the peripheral blood however and even an occasional cell arrested in the metaphase of mitosis.

Reticulocytes: No change was noted in the number of reticulocytes during the administration of the drug, but after its withdrawal, there was a marked rise in the reticulocytes although they did not attain the levels seen in the rabbit.

Bone Marrow: The marrow at first appearance gives the impression of red cell hyperplasia. A comparison with the number of reticulum cells shows that the red cell elements have remained constant while the white cell elements have undergone a decrease. In conjunction with the changes observed in the peripheral blood, this can be interpreted as *a selective decrease in the myeloid elements*. The decrease amounts to approximately $\frac{1}{3}$ of their initial values. The normoblasts show a number arrested in mitosis. The white cell elements demonstrate a shift to the right with a preponderance of young myelocytes. There are numerous eosinophilic elements present although they are not prevalent in the peripheral blood.

Histo-pathologic Studies:

There was no constant finding at gross autopsy. Some evidence of blood stasis was found. In one animal, there was a pleural effusion of moderate degree. The microscopic examination of the main organs showed no changes characteristic of colchicine poisoning although there was some lung pooling of blood.

Side Effects: During the course of administration of the drug, the rabbits were observed to lose 100 to 150 grams per week. They rapidly returned to their original weights after cessation of the drug. The cats

showed a greater reaction losing as much as 500 grams in one week. The weight loss in the cats however was dependent on the anorexia which occurred in 2 of the cats. The rabbits were closely watched for signs of diarrhea, but none appeared. The cats on the other hand exhibited all the classic signs of colchicine poisoning. They had diarrhea, painful muscles and skin, hyperpyralism, and inability to swallow. When fluid was forced into their mouth, it produced bouts of nausea and vomiting. It became necessary to sustain the two cats in Group 2 on sub-cutaneous glucose and saline solutions, but the symptoms disappeared after discontinuing the drug. The two cats in Group 3 showed only slight symptoms, namely diarrhea and when the dosage was dropped to $\frac{1}{4}$ mg. per kilogram of body weight, the side symptoms cleared up although the blood picture was maintained. *One of the cats also showed loss of hair on his neck for an area of approximately 10 centimeters square.* One of the most interesting side effects was noted in the rabbits. During the experiment, they had been kept in separate cages, but subsequently they were placed in a common cage. The females then all produced offspring. *In the untreated females the average litter was 5 to 7, but in the females who had previously been treated, it averaged only 2.*

IV. DISCUSSION AND CONCLUSIONS

The results will be discussed as a whole although there were some differences in the reaction of the cats from that of the rabbits which will be gone into later. Essentially however the findings were similar which justifies taking them together. With this drug, similar to that observed by Levison (12) with normal colchicine, *there is a quite constant latent time prior to the effect of the drug on the blood picture after administration lasting 72 hours.* Whether this is due to the formation of an intermediate compound being formed in the body prior to action as suggested by some authors, or whether it is due to that amount of time prior to the effect becoming manifest is not clear. However, it was interesting to note that when the method of administration bypassed the liver, i. v. in the rabbits, and intraperitoneally, the latent period was of this duration. *If the drug was given by mouth, in the case of two rabbits, the latent time was increased to 14 days,* indicating that some detoxifying process occurred in the liver. If an intermediate product was necessary prior to action, and it is logical to assume that the liver would be the place of its formation, this observation would tend to discredit the necessity of its formation. This suggests another line of thought, namely if the drug is detoxified in the liver, then how would its action be affected in its use with a patient having a deficiency in liver function. This would suggest further work along these lines and also the advisability of per-

ming liver function tests on any patient undergoing such therapy. No changes were observed macroscopically or microscopically on the animals who died, so no further clues are given. After discontinuation of the drug, a delay of 8 to 10 days was required until the initial blood leucocyte values were attained, which is in agreement with the observations of Beck (3) with ordinary colchicine.

The most important observation made in this experiment was that the neutrophils were sharply decreased while the other elements were not particularly altered so long as toxic doses were not used. At first, this does not seem to agree with the results of the workers with normal colchicine who reported after an initial leucopenia, a leucocytosis. However, it must be kept in mind that they were otherwise working with single high doses or doses much smaller than those used in this series, in order to prevent the death of their animals from direct toxicity. When they used a single high dose, their maximum concentration was at the initial stage and this blood level would depress the white cell elements similar to the effect observed here. Afterwards the drug would reach lower levels and a stimulatory effect would be produced resulting in the leucocytosis they recorded. In the series where they used smaller doses over a long period of time, again of necessity they could not approach the levels used here, so were often in a stimulating rather than the desired depressing range.

It was necessary to determine where this range was with the new drug. A surprising difference in the reaction of the rabbit and the cat were noted in this respect. The rabbit showed an optimal depression with a dosage of 3 mg. per kilo of body weight, but the cat proved to be much more sensitive and reached its optimum with a dose of $\frac{1}{4}$ mg. per kilogram of body weight. This difference in sensitivity has often been noted in experimental series for the rabbit, but the results with the cat are more pertinent for humans. At one stage the cats were on a dosage of $\frac{1}{2}$ mg., but developed such severe toxic effects that the dose was dropped to $\frac{1}{4}$ mg. where they quickly improved while the blood changes were maintained. This would suggest a similar upper limit for use in humans. It was only with the high toxic doses that significant depression of cell elements other than the neutrophils occurred, and therefore this suggested the use of small doses of the drug in myelogenous leukemia, where such a selective action is desired, see the clinical results of Moeschlin, Meyer and Lichtman (18).

Studies of the bone marrows showed that the mode of action of the drug was on the marrow with subsequent changes reflected in the peripheral blood. The white cell elements showed a definite depression in number and an increase in large early myelocytes and disappearance of most other mitosis of the cells in the metaphase. This was easily reversible after the drug was discontinued and provided a therapeutic advantage. The question arises as to why the red elements were not also depressed

since they also have mitosis. There were occasional arrested red elements observed in the peripheral blood, but no profound change other than that was seen. Is it due to a greater resistance of the red elements to the action of the desmethylcolchicin, or is it due to the longer lifetime of the red elements? A partial answer to this is that the animals were maintained on the drug for over a month with no subsequent change during that time or in follow up studies. This would indicate that it was not just a matter of sufficient time for the red elements to also be affected. In any case if such were to be found, the quick reversibility of the drug would merely necessitate periodic pauses in the therapy.

It is to be remembered that although many unpleasant side effects have been removed due to the lower toxicity of the drug, it is still a mitotic poison and any other cells undergoing mitosis may be similarly affected if the dose is increased or the cells are especially sensitive as in bone marrow, spermiogenesis, or ovulation. This is known in other cytostatic agents as aminopterin, nitrogen-mustard, urethane, etc. (17, 19). A control biopsy was performed on one of the male rabbits and compared with that of the animals who had died under therapy. There was a distinct *inhibition of the spermiogenesis* in agreement with the results of *Guieysee-Pelissier* (8) for ordinary colchicine. The *hair loss* of one animal can also be explained on this basis since similar effects are seen with the other mitosis poisons. These were also only transient remitting after the drug was discontinued. They should be looked for in patients receiving this therapy and told in advance about the hair loss.

The possibility that the cats in this series were merely showing blood changes due to an infectious agranulocytosis of virus origin is easily ruled out by the fact that there was an endemic source in the laboratory which infected all the animals shortly after their arrival. They were studied during the course of the illness, including sending the blood for virus studies. After recovery, which insures lifelong immunity, they were followed for an additional control period of 2 months before the experiment were begun.

The results which were obtained in this experiment showing a quick therapeutic onset after administration coupled with complete reversibility warrant its clinical trial. Its selective action on the myelogenous elements in the bone marrow with the almost complete exclusion of other elements as well as the lymphocytes has proved of great clinical value for the therapy of chronic and some acute myelogenous leukemias. See *Moeschlin, Meyer and Lichtman and Bock et al.* (28).

V. SUMMARY

1. A study was made to determine the effect of a new colchicine derivative, desmethylcolchicine (=Demecolcine Ciba), on the bone marrow and peripheral blood of 17 rabbits and 6 cats, as well any possible side effects.

2. The experimental results indicate *that desmethylcolchicin has an almost selective depressing effect on the myelogenous elements in the bone marrow which is quickly reversible*. A latent period of 3 days is required prior to its manifestation when given parenterally, and 12 to 14 days when given orally. The effect is reversed within 8 to 10 days after discontinuation of the drug. The toxic side effects were minimal when doses of 3 mg. per kilogram of body weight for the rabbits, and $\frac{1}{4}$ mg. per kilogram of body weight for the cats were administered. These side effects were arrest of spermiogenesis and some loss of hair in the rabbits.

These conclusions were based on the following criteria: bone marrow studies, absolute leucocyte counts and differential counts, erythrocyte counts, hemoglobin determinations, thrombocyte studies, and variations in the weight of the animals.

3. The experimental results and other clinical and experimental studies were discussed.

4. On this basis, the use of desmethylcolchicine is recommended for further clinical trial in the treatment of myelogenous leukemias.

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CURRICULUM VITAE

I was born in Brooklyn, New York on December 24, 1925, but have been a lifelong resident of Stamford, Connecticut. I studied at the Stamford High School and at the New York University, from which institution I received my A. B. degree in October, 1947. I commenced my medical studies at the Faculty of Medicine of the University of Zurich in 1948 and completed the course in 1953.

